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# REVIEW ARTICLE

# Heat shock and the heat shock proteins

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## Introduction

As all biologists appreciate, there is constant interaction between life and the environment, and temperature plays a critical role. It establishes distribution limits, it affects rates of function as well as the survival of organisms. Notoriously sensitive to temperature is plant growth. Critical steps in plant life such as germination, flowering and breaking of dormancy can be manipulated by the application of certain temperature treatments.

Animal life is mainly limited to a narrow range of temperatures, from a few degrees below the freezing point of water to approximately 50 °C. Animals nevertheless differ in the range of temperatures that they can tolerate. Temperature tolerance may however change with time and a certain degree of adaptation is possible. The limits of temperature tolerance for a given animal are not fixed. Indeed, it has been known for some time that exposure to a near lethal temperature often leads to a degree of adaptation so that a previously lethal temperature is tolerated. This particular response to heat shock has attracted considerable attention from molecular biologists over the last decade, which has resulted in a rapid accumulation of data providing considerable insights, not only into the molecular basis of acquired thermotolerance, but into stress physiology in general. The heat shock response is now known to occur in bacteria and in plants as well as in animals, and is a rapid but transient reprogramming of cellular activities to ensure survival during the stress period, to protect essential cell components against heat damage and to permit a rapid resumption of normal cellular activities during the recovery period.

## The heat shock proteins

Molecular biological interest in the heat shock response was really kindled in 1962 by a report (Ritossa, 1962) that brief heat treatment of *Drosophila hydei* larvae induced dramatic alterations in gene activity as judged by the changes in 'puffing' patterns observed in the salivary gland polytene chromosomes. However, it was some time until it became clear that a main feature of the heat shock response from bacteria to man (Schlesinger *et al.*, 1982) is the vigorous but transient activation of a small number of specific genes previously either silent or active at low levels. New mRNAs are actively transcribed from these genes and translated into proteins which are collectively referred to as the heat shock proteins, or hsps (see Table 1).

The optimum temperature range of hsp induction varies considerably with the organism, but relates to the physiological range of supraoptimal temperatures within which active adaptation is observed. For example, this is around 40–50 °C for birds and mammals, 35–37 °C for *Drosophila*, 33–35 °C for yeast and 35–40 °C for plants. However, it has been found that the optimum can vary between different cell types of a single organism and between individual hsps from even one cell type.

Hsps were first reported in *Drosophila* by Tissières and his colleagues (Tissières *et al.*, 1974). The exact number of different types of hsp varies considerably in different organisms and cell types, but in all cases proteins of approx. 84 and 70 kDa (hsp 84 and hsp 70) are amongst the most prominent. A dramatic feature is that these particular proteins have been highly conserved in evolution. For example, the hsp 70 proteins of *Drosophila* and yeast have a 72% amino acid identity (Ingolia et al., 1982), and their hsp 84 proteins have a 63% identity (Hackett & Lis, 1983; Farrelly & Finkelstein, 1984). The derived protein sequence of a human hsp 70 gene is found to be 73% homologous to *Drosophila* hsp 70 (Hunt & Morimoto, 1985) and that from maize is 68% homologous (Rochester *et al.*, 1986).

Most organisms also produce small hsps of 15–30 kDa. However, whereas *Drosophila* cells induce four very closely related proteins of 22, 23, 26 and 28 kDa, yeast appears to produce only one at 26 kDa. These smaller hsps are not so well conserved in evolutionary terms, although nucleic acid sequence analysis has demonstrated certain homology amongst the small hsps of insects, vertebrates and nematodes (Bienz, 1984*a*; Russnak *et al.*, 1983), as well as a part homology to α-crystallin (Craig *et al.*, 1982).

A problem when evaluating the hsp spectrum of an organism by two-dimensional electrophoresis has been the complexity due to the presence of 'isoforms' of the major hsps (Slater *et al.*, 1981; Kioussis *et al.*, 1981; Welch *et al.*, 1983). This would appear to be the result of multigene families as well as extensive covalent modifications, e.g. phosphorylation (Kim *et al.*, 1984), methylation (Wang *et al.*, 1981) glycosylation (Kelley & Schlesinger, 1978), or ADP-ribosylation (Carlsson & Lazarides, 1983). Such modifications may affect both the function and metabolic stability of hsps. It is known that their metabolic stability can vary and can be influenced by nutritional status (Lanks, 1983).

In very general terms, if the predominant hsps in eukaryotes are considered, they all appear to be acidic (pI 5.0–6.5) and to fall into two major groups: (*a*) 68–110 kDa and (*b*) 15–30 kDa. In order to examine the properties of particular hsps in more detail, purification procedures have been reported (Welch & Feramisco, 1982) for the mammalian 'hsp 70 class' proteins. A recent rapid technique is based on their ATP-binding properties and involves a two-step procedure employing DE52 ion-exchange chromatography followed by affinity chromatography on ATP–agarose (Welch & Feramisco, 1985). A problem revealed recently in studies on hsp 70

Abbreviations used: hsp(s), heat shock protein(s); HSE, heat shock element; HSTF, heat-shock transcription factor.

**Table 1. Heat shock proteins of eukaryotes**

For references see the text and Ashburner & Bonner (1979), Schlesinger *et al.* (1982), Neidhart *et al.* (1984), Craig (1985), Beinz (1985) and Key *et al.* (1982).

| Class | Species | Size range (kDa) | Covalent modifications | Intracellular location | Comment |
|---|---|---|---|---|---|
| hsp 110 | Mammals | 110 | | Nucleolus | Normal nucleolar component |
| hsp95 | Vertebrates | 92–105 | Phosphorylation | Golgi | Normal cell component identical with membrane proteins induced by glucose deprivation |
| | Plants | 95 | Phosphorylation | | |
| hsp 84 | Vertebrates | 83–90 | Methylation, phosphorylation and ADP-ribosylation | Cytosol | Normal cell component; associates with steroid receptors |
| | Yeast | 84 | | | |
| | *Drosophila* | 84 | | | |
| | Plants | 80 | | | |
| hsp 70 | All | 68–74 | Phosphorylation | Cytoplasm, cytoskeleton; migration to nucleus and nucleolus after heat shock | Mainly constitutive cell proteins; *E. coli* equivalent is the *dnaK* protein |
| hsp 60 | Plants | 60 | | Mitochondria | Encoded by mitDNA |
| Small hsps | Vertebrates | 23–30 | Glycosylation | | |
| | *Drosophila* | 22 | | Nuclear matrix after heat shock | |
| | | 23 | | | |
| | | 26 | | | |
| | | 28 | | | |
| | Yeast | 26 | | | |
| | Plants | 15–27 | | Aggregate after heat shock to give cytoplasmic granules | Hsp 21, hsp 22 transported to chloroplasts |

class proteins is degradation *in vitro* (Mitchell *et al.*, 1985). This occurs even during electrophoresis and does not appear to be mediated by a general proteinase, but rather the hsp 70s have a slow autoproteolytic action.

During investigation of hsp induction it became clear in mammalian cells that particular hsps such as hsp 28, hsp 72, hsp 73, hsp 84, hsp 90, hsp 100 and hsp 110 were all transiently synthesized at an elevated rate after heat stress. However, with the possible exception of hsp 72, all were present at significant levels in unstressed cells at 37 °C (Welch *et al.*, 1983). Thus in the mammalian 'hsp 70 class' a distinction can be drawn between the 'constitutive' hsp 73 and the 'inducible' hsp 72. Peptide mapping shows that the hsp 73 and hsp 72 proteins are similar but not identical (Welch & Feramisco, 1984).

A third type of proteins that also appear to be related to the 'hsp 70 class' was detected in mouse cells and are synthesized constitutively but are *not* elevated upon heat shock (Lowe & Moran, 1984). Such proteins have been referred to as 'heat shock cognate proteins' and are also detectable in *Drosophila* (Ingolia & Craig, 1982). The possibility that the 'constitutive' group also contains these 'cognate' species remains to be determined. Present separation techniques do not yet permit a clear distinction.

The response of bacteria to 'shift-up' in growth temperatures has also been extensively studied. In some aspects the response resembles that in animal and plant cells. Almost immediately after an elevation in growth temperature in *Escherichia coli* there is an accelerated synthesis of 17 or so polypeptides (Neidhardt *et al.*, 1984). Each of these proteins exhibits a characteristic increase in its rate of synthesis upon temperature shift. In general when cells are shifted from 30 °C to 40 °C rates of synthesis increase between 5- and 20-fold. The increased rates of synthesis reach a peak 5–10 min after the shift-up and then decline to a near steady-state somewhat greater than those at the low temperature. A particularly important aspect of this transient response is that one of the *E. coli* hsps, the product of the *dnaK* gene, is around 45–50% homologous to the hsp 70 proteins of human, *Drosophila* and yeast (Hunt & Morimoto, 1985; Craig *et al.*, 1982; Bardwell & Craig, 1984). In *E. coli*, at least, this response appears to be regulated by the protein product of a gene *htp* R (Neidhardt & Van Bogelen, 1981) [or *hin* (Yamamori & Yura, 1982)] which exerts an overall positive control on the unlinked operons of the heat shock regulon.

### Mechanisms underlying hsp gene transcription

The induced synthesis of hsps in mammalian cells for example can be brought about by a variety of agents other than heat (see Table 2). This makes it difficult to be clear about the mechanisms responsible for triggering the induction process. Many inducers such as heat (Slater *et al.*, 1981) ethanol (Li, 1983), transition series

**Table 2. Heat shock protein synthesis in mammalian cells**

References: [a]Burdon *et al.* (1982), [b]Li (1983), [c]Thomas & Mathews (1984), [d]Sciandra & Subjeck (1983), [e]Li & Schrieve (1982), [f]R. H. Burdon & V. Gill, unpublished work.

| Inducers | Non-inducers |
|---|---|
| Heat [a] | Cyclic AMP [a] |
| | Butyrate [a] |
| Ethanol [b] | Dimethyl sulphoxide [a] |
| Sodium arsenite [a] | Phorbol esters [f] |
| Heavy metals (e.g. $Cd^{2+}$) [a] | Colchicine [f] |
| Amino acid analogues [c] | Cytochalasin B [f] |
| | Cycloheximide [f] |
| | Azacytidine [a] |
| Glucose starvation followed by refeeding [d] | Ouabain [f] |
| | Calcium ionophore [f] |
| | Amiloride [f] |
| Anoxia followed by oxygenation [e] | $H_2O_2$ [f] |
| | NaCn [f] |
| | NaF [f] |
| | Dinitrophenol [f] |
| | Azide [f] |

metals (Burdon *et al.*, 1982; Levinson *et al.*, 1980), sodium arsenite (Burdon *et al.*, 1982) thiol reagents (Burdon *et al.*, 1982; Levinson *et al.*, 1980) and amino acid analogues (when incorporated into protein) (Thomas & Mathews, 1984) might be expected to denature or damage some proteins, or yield altered cellular proteins. On the other hand, sudden increases in metabolic activity such as would follow refeeding of glucose after starvation (Sciandra & Subjeck, 1983) or reoxygenation following hypoxia (Sciandra *et al.*, 1984; Li & Schrieve, 1982) might conceivably cause protein damage through increased metabolism and subsequent oxygen-related free radical activity.

A key point when considering possible induction mechanisms is the fact that the basic mechanism whereby this is achieved appears to be highly conserved in evolution. For example, when hsp 70 genes from *Drosophila* are introduced into mouse cells, monkey cells, sea urchin embryos, *Xenopus* oocytes, yeast or tobacco tissue, they are only actively transcribed when the recipient cells are subject to hyperthermia (Pelham, 1982, 1985; Corces *et al.*, 1981; Bienz, 1984*b*; Voellmy & Rungger, 1982; McMahon *et al.*, 1984; Ayme *et al.*, 1985; de Banzie *et al.*, 1986; Spena *et al.*, 1985). This experimental approach has been developed to assess the effects of DNA sequence deletions so as to determine the position and nature of the DNA sequence elements responsible for regulating the induction of hsp gene transcription (Pelham, 1985; Ayme *et al.*, 1985). In the case of the most well studied hsp gene, the hsp 70 gene from *Drosophila*, a short special sequence has been identified that is required for transcription in heat treated monkey (COS) cells (Pelham, 1982). This special sequence is located about 20 nucleotides upstream of the 'TATA box', the conserved element found in all eukaryotic gene promoters. When this special sequence is compared with the sequences upstream of other *Drosophila* hsp genes, homologies are apparent with the minimal consensus sequence being the hyphenated dyad C--GAA--TTC--G. This has been referred to by Pelham (1985) as the heat shock element, or HSE (see Fig. 1). A similar sequence has been detected adjacent to hsp genes of some other species, including plants (Schöffl & Baumann, 1985), and may well be the basis for the successful activation of the *Drosophila* hsp genes in the cells of other species. Although induction seems to be most efficient when the HSE is closest to the TATA box, the distance can vary. For example, the *Drosophila* and *Xenopus* hsp 70 genes have HSEs 74 and 14 nucleotides from the TATA box but yet they work with equal efficiency (Pelham, 1985; McMahon *et al.*, 1985). In the soybean heat shock gene, hs6871 (Schöffl & Baumann, 1985), the consensus element is some 28 nucleotides from the TATA box.

What then is the role of the HSE? Present evidence suggest that it is a protein binding site. From digestion experiments of *Drosophila* chromatin with DNAase I, followed by exonuclease, it has been possible to establish that the TATA box sequence is protected by protein, both before and during heat treatment, whereas the HSE is only protected by protein during the heat treatment (Wu, 1984). Transcription of hsp genes appears to correlate with the presence in *Drosophila* nuclei of an HSE-binding protein (Parker & Topol, 1984). This has now been partially purified and shown to bind to the HSE of the hsp 70 gene and to be necessary for the transcription *in vitro* of *Drosophila* hsp genes but not for other genes. It is now referred to as a heat-shock transcription factor, or HSTF. Additional experiments have identified a second protein fraction that stimulates transcription from a wide selection of genes and can protect the TATA box and transcription initiation site from DNAase. This factor, plus HSTF under heat shock conditions, effectively cover around 130 nucleotide pairs of the hsp 70 gene promoter (Parker & Topol, 1984), and thereby include the sequences that are known to be required for hsp gene promoter function (at least in *Drosophila*).

A complete human hsp 70 type gene has now been characterized. It is 2440 nucleotides in length, comprising a 5′ non-coding sequence of 212 nucleotides and a 3′ non-coding region of 242 nucleotides, and thus encodes a protein of 69800 Da (Hunt & Morimoto, 1985). Upstream of the 5′-terminus are the TATA box (at −30) and the dyad sequence CTGGAATATTCCCG (at −79) that shows considerable homology with the consensus HSE sequence common to the *Drosophila* hsp genes. At the nucleotide level the human and the *Drosophila* genes are 72% identical whereas the human and *E. coli dnaK* genes are 50% identical. This is of considerable evolutionary interest as the genes are more highly conserved than is necessary, considering the degeneracy of the genetic code. This lack of accumulated silent nucleotide substitutions has led Hunt & Morimoto (1985) to propose that there may be additional information in the nucleotide sequence of the hsp 70 genes (or their corresponding mRNAs) that precludes the maximum divergence allowed in the silent codon positions.

Having identified HSTFs and their binding to HSEs, a question remains as to how this is regulated. It turns out that HSTF can be extracted from both heat-treated and control *Drosophila* cells, but it appears to be less effective as a transcription factor when extracted from the unheated cells (Parker & Topol, 1984). It may be modified in some way following hyperthermia. This might be achieved through denaturation, or other

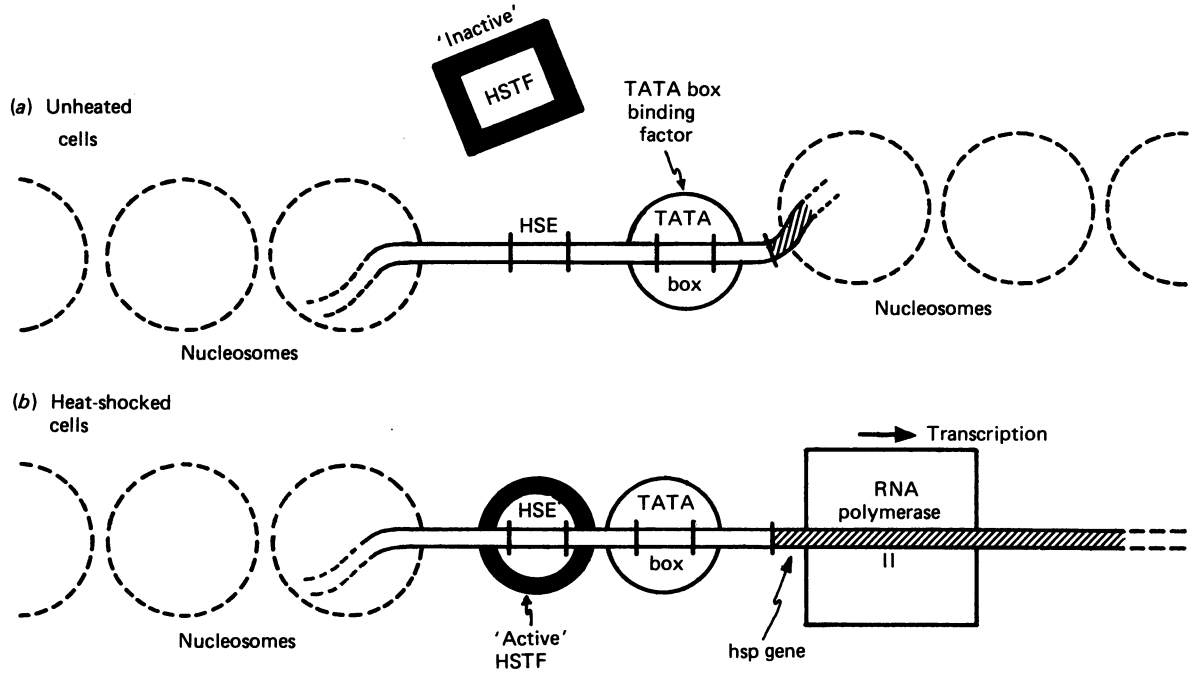


**Fig. 1. A possible mechanism involving transcription factors whereby hsp genes might be activated**

The regulatory DNA sequences, HSE (heat shock element) and TATA box, lie upstream from the hsp gene in a region free of nucleosomes. In unheated cells (*a*) nuclease sensitivity studies indicate that the TATA box binding factor is bound to the TATA box sequence whereas the HSE element is free of protein. After heat shock (*b*) there is some specific structural alteration to the heat shock transcription factor (HSTF) that allows it to bind to the HSE sequence. This allows the transcription of the hsp gene by RNA polymerase II.

damage, by agents known to induce hsp synthesis. However it is unlikely, although not impossible, that all these treatments could affect such a single 'sensor' molecule in precisely the same way. A proposal has been that the intracellular accumulation of abnormal, damaged, proteins *per se* may trigger hsp gene transcription (Findley *et al.*, 1984); abnormal proteins certainly result when cells are treated with the amino acid analogues known to induce hsp synthesis (Thomas & Mathews, 1984). Moreover the presence of abnormal truncated actin molecules in cells of certain flightless *Drosophila* mutants results in the synthesis of hsps (Karlik *et al.*, 1984; Hiromi & Hotta, 1985) as does the injection of denatured proteins into frog oocytes (Ananthan *et al.*, 1986).

Normally, abnormal proteins are degraded within the eukaryotic cell by an ubiquitin-dependent degradation system (Hershko & Ciechanover, 1982; Hershko, 1984) (see Fig. 2). They are attached by the *N*-terminal (and lysine) amino groups to ubiquitin, a 76 amino acid protein, through a peptide bond, and become substrates for proteolysis. After treatment of Hela cells with heat or the other agents known to induce hsps, we find increases in degradation of intracellular proteins. Thus it may be that acute hyperthermia, or treatment with other hsp inducers, produces an increase in abnormal protein requiring ubiquitination (see Fig. 2) and this may lead to a transient shortage of free ubiquitin. A recent observation is that the gene for ubiquitin itself is activated upon heat treatment of both chick and yeast cells (Bond & Schlesinger, 1985) which would be expected to restore supplies of ubiquitin.

Does a transient ubiquitin shortage serve to induce hsp gene transcription by affecting the HSTF in some way? A temperature-sensitive mutant mouse cell line is known (ts 85) in which no ubiquitination is possible at the non-permissive temperature, yet hsps are synthesized (Findley *et al.*, 1984). Unfortunately our knowledge of the intracellular roles of ubiquitin is incomplete. It is quite clear that not all proteins that are ubiquitinated are degraded. Histone 2A is known to be normally extensively ubiquitinated. Nevertheless it has been observed that the level of ubiquitination of histone 2A is markedly reduced in *Drosophila* cells subject to hyperthermia (Glover, 1982). This has led to the proposal (Munro & Pelham, 1985) that particular proteins such as HSTF may, like histone, be normally maintained in an ubiquitinated form but with the ubiquitin moiety being continually removed and replaced. Treatments of the cell that cause a fall in the ubiquitin level, by producing an accumulation of damaged or abnormal protein substrate, might result in an increased level of HSTF in a non-ubiquitinated form (see Fig. 2). Such a form may be the 'active' form required to initiate hsp gene transcription. This has yet to be tested. An alternative possibility (see Fig. 2) is that following heat shock, or treatment with other inducers, there is direct alteration of HSTF structure (e.g. as an outcome of the increased proteolysis, or as a result of increased levels of free radicals). Another significant factor may be the noteable accumulation of non-histone proteins in nuclei and chromatin of mammalian cells following heat shock or ethanol treatment (Roti-Roti & Wilson, 1984). These may include activating proteins.

Transcriptional regulation after heat shock has also been investigated in *E. coli*. Cowling *et al.* (1985) have

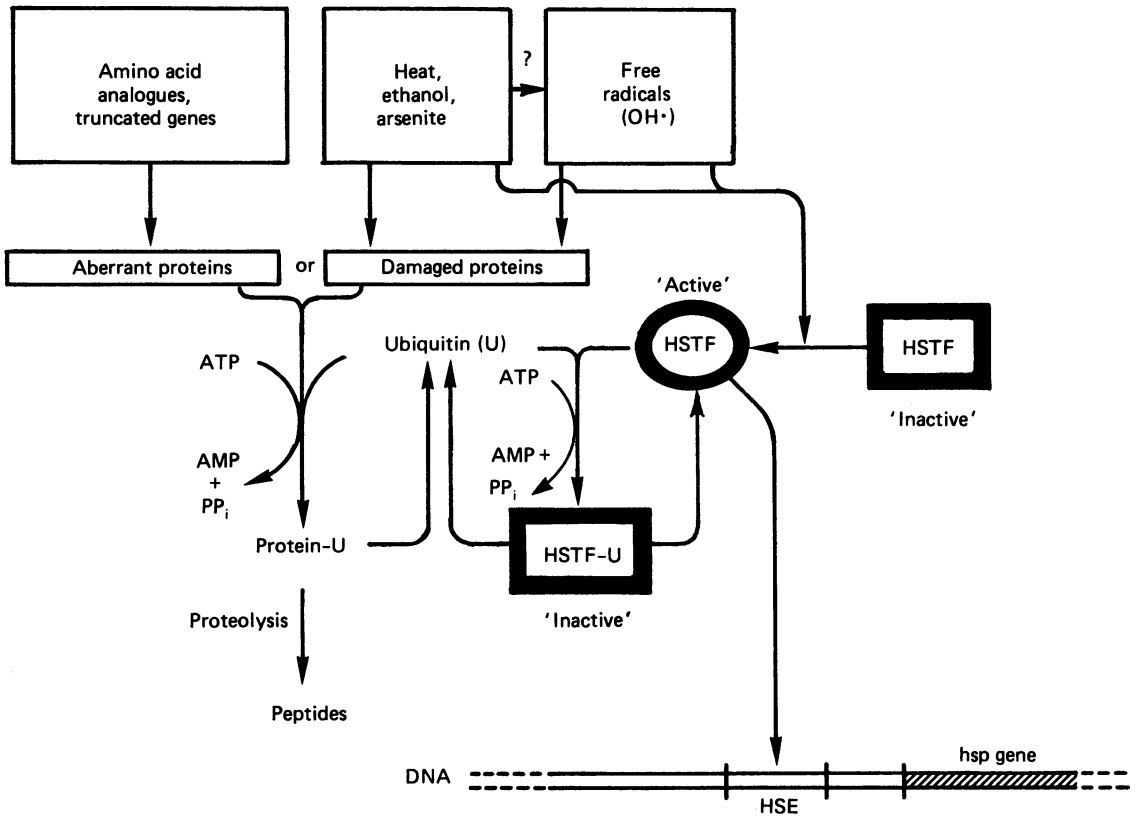


**Fig. 2. Possible means whereby the presence of damaged cellular proteins might signal the induction of hsp gene transcription**

The introduction of truncated genes into mammalian cells, or treatment with amino acid analogues, will result in the synthesis of aberrant proteins. Other treatments, such as heat or ethanol, may damage cell proteins by denaturation, or by mechanisms involving free radicals. Aberrant or damaged proteins are then recognized by the ATP-requiring ubiquitin-dependent degradation system. However, a suggestion is that the normally 'inactive' form of heat shock transcription factor (HSTF) is in an ubiquitinated state; thus the depletion of the cellular levels of free ubiquitin due to any increases in levels of aberrant or damaged protein will lead to a rise in the levels of non-ubiquitinated HSTF. This may be the 'active' form. Alternatively, other mechanisms which do not involve ubiquitin may bring about the structural alteration of 'inactive' HSTF to 'active' HSTF (e.g. denaturation, free radical damage, proteolytic cleavage).

identified promoters for the heat shock operons *dnaK* and *groE* as well as for the gene encoding the heat shock protein C62.5. Transcription from each promoter is heat-inducible *in vivo* and each is recognized *in vitro* by RNA polymerase containing $\sigma^{32}$ [the factor encoded by *htp* R (*rpo* H)] but not by RNA polymerase containing $\sigma^{70}$. Examination of the heat shock promoter regions indicates a consensus sequence having TNTCNCCCTTGAA in the −35 region and CCCCATTTA in the −10 region. These sequences differ from the consensus sequence recognized by RNA polymerase holoenzyme containing $\sigma^{70}$, the major $\sigma$ in *E. coli*. The mechanism whereby the critical *htp* R gene transcription is activated is however unclear, but aberrant proteins may also be the initial stimulus. Although there is no bacterial protein analogous to ubiquitin, certain of the heat-induced proteins are involved in degradation of abnormal proteins (e.g. the Lon protein).

An important feature to emerge from the bacterial work concerns the regulatory functions of one of the heat shock proteins, the *dnaK* gene product. Mutations in this product have been shown to affect both DNA and RNA synthesis in *E. coli*. Moreover, bacteria containing the *dnaK* 756 mutation fail to turn off the normally transient heat shock response at 43 °C. Instead they continue to synthesize the heat shock proteins in large amounts. The *dnaK* protein appears to be an inhibitor of the heat shock response in *E. coli* (Tilly *et al.*, 1983) and indicates that the induction of heat shock proteins is subject to 'autoregulation'. How this is achieved is uncertain, but *dnaK* protein has protein kinase activity (Zylicz *et al.*, 1984) and this might inactivate $\sigma^{32}$.

This notion of autoregulation is of general significance as it has been clear from work with systems other than bacteria (e.g. *Drosophila*, yeast, mammalian cells) that the transcriptional activation of hsp genes is a transient phenomenon (Di Domenico *et al.*, 1982; Slater *et al.*, 1981). In heat-shocked *Drosophila* cells it is clear that transcriptional regulation of hsp genes is dependent on accumulated levels of hsps themselves (Di Domenico *et al.*, 1982). A specific accumulation of functional hsp appears to repress hsp gene transcription. How this is achieved is not clear, but a number of studies indicate the migration of hsps to nuclei following heat shock (Velazquez & Lindquist, 1984; Welch & Feramisco, 1984), and proteins of the hsp 70 class have been found in association with heterogeneous nuclear ribonucleoproteins as well as with messenger ribonucleoproteins (Kloetzel & Bautz, 1982). Such associations may have an

important role in the autoregulation phenomenon, at least in eukaryotes.

**Other heat-shock-induced transcriptional changes**

Whilst considerable attention has been directed towards heat shock gene transcription and its regulation following heat shock, the transcription of certain other genes is also known to be affected. For example, in cultured *Drosophila* cells, non-hsp gene transcription is rapidly suppressed upon heat shock but returns to normal levels after cells are returned to normal culture temperatures (Findley & Pedersen, 1981). On the other hand, it is not clear how widespread is the suppression of non-hsp structural gene transcription following heat shock in other species.

More attention has been given to changes in transcription of genes for stable RNAs. Heat shock leads to extensive changes in nuclear ultrastructure (Simard & Bernhard, 1967; Warocquier & Scherrer, 1969; Heine *et al.*, 1971) and a pronounced inhibition of ribosomal RNA production in *Drosophila* cells (Ellgard & Clever, 1971; Spradling *et al.*, 1977), mammalian cells (Amalric *et al.*, 1969; Warocquier & Scherrer, 1969), yeast (Gorenstein & Warner, 1976; Kim & Warner, 1983) and plant cells (Dawson & Grantham, 1981; Fransolet *et al.*, 1979). The effects on heterogeneous nuclear RNA synthesis are less marked by comparison. For example, in Hela cells treated at 43 °C, Zieve *et al.* (1977) found 45 S ribosomal RNA precursor synthesis inhibited by 90%, heterogeneous nuclear RNA synthesis inhibited by only 15%, but tRNA and 5 S RNA stimulated by some 15%.

The heat-sensitive component in the ribosomal RNA synthetic process in hamster cells does not appear to be RNA polymerase I itself, as judged by assay *in vitro* (Bouche *et al.*, 1981). The processing of ribosomal RNA precursors is certainly heat-sensitive (Bouche *et al.*, 1981). It has been suggested that this may be as a result of their defective methylation (Bouche *et al.*, 1981) which might affect the processing mechanisms, or alternatively due to a deficiency of ribosomal protein brought about by the heat shock (Warner, 1982). Such proteins are necessary for the formation of preribosomes which are believed to be the actual substrates for the processing enzymes.

Despite the inhibitory effects of heat on ribosomal RNA synthesis the effect is reversed after shift back to normal growth temperatures. For example in Hela cells treatment at 45 °C, but only for 10 min, leads to an inhibition of synthesis of around 60%. After return to 37 °C, recovery is complete by around 2 h. The speed of this recovery depends on the severity of the initial heat treatment. Parenthetically, it should be mentioned that, following this recovery, the process appears to be extremely heat-resistant (R. H. Burdon, unpublished work).

**Translational control following heat shock**

Although heat shock can cause considerable changes in transcriptional patterns, effects specifically on translational control are no less dramatic. On the other hand, it is clear that different organisms achieve rapid shifts in protein synthesis in quite different ways.

Perhaps the most well studied organism in this connection is *Drosophila*, where heat shock induces a mechanism of translational control which both promotes the translation of hsp mRNAs and specifically represses the translation of pre-existing mRNAs (McKenzie *et al.*, 1975; Lindquist, 1980, 1981). The pre-existing mRNAs however remain stable and translatable in other systems *in vitro*, or *in vivo* following recovery from heat shock (Kruger & Benecke, 1981; Storti *et al.*, 1980). From experiments with whole cells, and with translation systems *in vitro*, two points of regulation appear important. Firstly, there appears to be a change in the translation mechanism that permits the exclusive recognition of hsp mRNAs and secondly a requirement for some specific structural feature present on hsp mRNAs that allow them to be recognized as such. Scott & Pardue (1981), from work on *in vitro* translation systems from heat-shocked *Drosophila* cells, found that addition of ribosomes from normal cells restored normal protein synthesis. A key question was whether the dephosphorylation of ribosomal protein S6 observed afer heat shock was the reason for the change in translational specificity. However, experiments with whole cells suggested that there was no correlation between the two phenomena (Olsen *et al.*, 1983). More recent work with mRNA-dependent *in vitro* translation systems from normal and heat-shocked *Drosophila* cells that can be fractionated into ribosomes and supernatant fractions, as well as reconstituted from these fractions, suggests that the specificity for the protein synthetic patterns after heat shock is due to supernatant factors, rather than ribosomes *per se* (Sanders *et al.*, 1986). With regard to special structural features of hsp mRNAs, it has been known for some time that the 5′ untranslated regions of *Drosophila* hsp mRNAs are quite long. For example, in mRNAs for hsp 70 such a region is 244 nucleotides. Moreover, this sequence is unusually rich in adenine residues (46%) (Ingolia & Craig, 1981). In recent experiments (Klemenz *et al.*, 1985; McGarry & Lindquist, 1985) various fusion genes were constructed using elements of the hsp 70 gene of *Drosophila* and the gene for alcohol dehydrogenase. From transfection experiments using various gene constructs, the specific feature responsible for the preferential translation of hsp mRNAs is not yet clear, but nevertheless resides in the 5′ untranslated region.

In yeasts the situation is quite different from that encountered in *Drosophila*. Yeast cells do not possess a special mechanism for sequestering pre-existing mRNAs from translation. Instead most of these mRNAs simply disappear rapidly from the cell whilst those that are retained continue to be translated (Lindquist, 1981).

In mammalian cells the situation is different but in yet another way. On one hand they are somewhat like yeast in as much as there is no extensive sequestration of pre-existing mRNAs from translation. Unlike yeast, however, the pre-existing mRNAs do not disappear (Slater *et al.*, 1981; Kioussis *et al.*, 1981). When Hela cells for example are subjected to hyperthermia an immediate effect is a marked inhibition of total protein synthesis and decay of polysomes. Nevertheless, when the heat treatment is continuous at temperatures less than 42 °C, protein synthesis recovers to reach levels higher than in untreated cells. After acute, but brief (10–15 min) hyperthermia (43–46 °C), the polysome profile is restored and protein synthesis recovers (to levels between 150 and 200%) if the temperature is returned to 37 °C (Burdon *et al.*, 1982). These recovery processes are

partly inhibited if actinomycin D (0.5 $\mu$g/ml) or puromycin (200 $\mu$g/ml) are added.

Possible reasons for the initial inhibition and loss of polysomes include (*a*) the inactivation of eIF-4F function (Panniers *et al.*, 1985), (*b*) the dephosphorylation of ribosomal protein S6 and the phosphorylation of protein L14 which are observed during the initial phase of heat shock (Kennedy *et al.*, 1984), (*c*) the activation of a protein kinase analogous to the haem-controlled repressor of reticulocytes and the subsequent phosphorylation of initiation factor eIF-2 (de Benedetti & Baglioni, 1986), or other initiation factors (Duncan & Hershey, 1984). Although eIF-2 phosphorylation appears to limit mRNA translation in heat-shocked cells (de Benedetti & Baglioni, 1986) we find the addition of purified eIF-2 to lysates from heat-shocked cells has a marked stimulatory effect on protein synthesis *in vitro* (R. H. Burdon, unpublished work). However, addition of fresh ribosomal subunits to such lysates also had restorative effects. Thus it may well be that the phosphorylation of eIF-2 as well as alteration to other initiation factors and ribosomal subunits are all contributory to the initial inhibition effects.

Having discussed possible contributory factors to the initial inhibition of protein synthesis, the problem of recovery after heat shock remains. During the recovery period in Hela cells the phosphorylation of ribosomal protein S6 is partly restored whereas that of ribosomal protein L14 remains unchanged (Kennedy *et al.*, 1984). In addition, phosphorylation of eIF-2 is reduced (de Benedetti & Baglioni, 1986). However these observations do not account for the partial requirement for RNA and protein synthesis suggested from the experiments using low levels of actinomycin D and protein synthesis inhibitors. Thus, although a readjustment of covalent modifications in the protein synthetic apparatus may be of considerable importance, there may for example be an additional need to replace some damaged ribosomes and initiation factors.

**Heat shock proteins and their developmental regulation**

A recent advance in our understanding of the heat shock response is that superimposed upon the transient heat-induced activation of hsp genes just described, there are clear indications of developmental regulation. For example, there are developmental stages, or specific cell types, in which certain hsp genes are active under normal physiological conditions and others in which certain hsp genes are not inducible, even after heat shock.

During ascospore development in yeast (Kurtz *et al.*, 1986), hsp 26 and hsp 84 were strongly induced whereas the major hsp 70 was neither induced nor inducible. Instead, two proteins related to hsp 70 were induced. This is similar to the situation in normal oogenesis in *Drosophila*. In adult females RNAs for hsp 26, hsp 28 and hsp 84 are induced in ovarian nurse cells and passed into the developing oocyte. There is no observable accumulation of hsp 70 mRNA. In fact, in late egg chambers and early embryos hsp 70 is not induced even with heat shock (Zimmerman *et al.*, 1983). During embryonic development hsp 84 mRNA continues to be present at most stages in non-heat-shocked animals. Hsp 68 and hsp 70 mRNAs are also present at very low levels but at higher levels in pupae. Hsp 23 and hsp 27 mRNAs, although barely detectable in early third instar larvae, are major components of late third instar and early pupae RNA. Later in development the smaller hsp mRNAs decrease, although there is a subsequent increase of hsp 26 and hsp 27 mRNAs in adult females (Mason *et al.*, 1984).

In the mouse embryo, activation of the embryonic genome occurs at the late two-cell stage and results in a dramatic change in the proteins synthesized. Amongst the two-cell-specific proteins are two proteins identical to hsp 68 and hsp 70 (of the mouse 'hsp 70 class') (Bensaude *et al.*, 1983). Mouse embryonal carcinoma cells, as well as embryonic ectoderm cells, also express high levels of hsps in the absence of stress (e.g. hsp 89, hsp 70 and hsp 50) (Bensaude & Morange, 1983). Differentiation *in vitro* induced by retinoic acid and dibutyryl cyclic AMP was accompanied by a decrease in the spontaneous abundance of hsps. In certain mouse embryonal carcinoma cells it was also clear that the usual heat induction of hsps did not occur, although heat inducibility of hsp 68 can be restored after differentiation *in vitro*. Similarly, in the early mouse embryo hyperthermia does not induce hsp 68 at the eight-cell stage but does so at the blastocyst stage (Morange *et al.*, 1984).

Developmental regulation is also clear in *Xenopus*; however, the only cells that contain hsp 70 mRNA without heat shock are oocytes. After fertilization this mRNA becomes undetectable, but appears as a heat-inducible mRNA at gastrulation and developmental stages thereafter. In contrast, hsp 30 mRNA is not detectable even after heat shock in oocytes and embryos, although heat inducibility does appear at the tadpole stage (Bienz, 1984*a*). Even in adult cells there are examples of hsp gene repression. For example, chicken reticulocytes respond to heat with the induction of only one hsp (hsp 70) whereas lymphocytes induce the synthesis of hsp 70, hsp 89, hsp 23 and hsp 22 (Morimoto & Fodor, 1984).

How then are hsp genes non-co-ordinately activated during normal development? What mechanisms cause their 'repression' or 'non-heat inducibility'? One possibility is that limiting amounts of 'activated' HSTF (specific for certain cell types) combined with variable strengths of individual promoters, determine which genes are activated at low or high temperature (Bienz, 1985). For instance, it is now known that the promoter for hsp 83 of *Drosophila* is strong and has three overlapping HSEs, the proximal one being only 16 nucleotides from the TATA box. Thus the gene may be inducible at extremely low 'active' HSTF concentrations, such as might occur in unstressed cells. Other hsp genes have less strong promoters. Where they contain two adjacent HSEs, they are either further away from the TATA box, or one is only a weak binding site (Bienz, 1985). An alternative possibility is that there are other cell-specific factors which recognize promoter elements distinct from HSE and which are only present adjacent to certain hsp genes. In *Xenopus* oocytes, the constitutive expression of hsp 70 may be due to the recognition by an oocyte-specific factor of a conserved hexanucleotide on the 3′ side of the HSE, which facilitates the binding of 'inactive' HSTF to HSE, thus promoting hsp 70 gene transcription (Bienz, 1985). In *Drosophila* it is known that activation of certain small hsps occurs in response to the moulting hormone ecdysone (Vitek & Berger, 1984), thus suggesting the ecdysone receptor as an additional regulatory factor. Sequences resembling the steroid

receptor binding site may overlap with HSEs (see Bienz, 1985). From experiments in which hsp 26 deletion variants of hsp 26 gene have been introduced into the *Drosophila melanogaster* germ line, it seems that there are separate regulatory elements for the 'heat-inducible' and 'constitutive' ovarian expression of that particular hsp gene (Cohen & Meselson, 1985). A similar approach, but using hsp 26–*lac* Z fusion genes, histochemical assay for $\beta$-galactosidase and *in situ* hybridization to probes for hsp 26 mRNA, revealed hsp 26 expression in numerous tissues during development. However constructs with different lengths of upstream sequences showed tissue-specific expression (Glaser *et al.*, 1986).

Recent work with cultured human cells, which is also very relevant to developmental regulation, demonstrates that mitogenic stimulation by serum addition will induce the increased transcription of hsp 70 genes (Wu & Morimoto, 1985) and deletion analysis of 5′ flanking sequences indicates that the human hsp 70 promoter contains at least two regulatory domains: (i) a distal domain, between −107 and −68, responsive to heat shock containing the sequence CTGGAATATTCCCG which, as already mentioned, shows considerable homology to the consensus *Drosophila* HSE sequence; (ii) a proximal domain, responsive to stimulation by serum with a purine-rich sequence GAAGGGAAAAG at −58 (Wu *et al.*, 1986).

**Heat shock and development**

A question that these observations on developmental regulation raise is whether there is a connection *in vivo* between stress and developmental regulation. (Zucker *et al.* (1983) demonstrated a set of genes in *Dictyostelium* which can be induced in response to heat shock or to the stresses that trigger the initiation of development. They were able to show the principal component of this 'stress' was the high density of the cells.

Geneticists working with *Drosophila* have employed heat shock experimentally to produce developmental defects (Mitchell & Lipps, 1978). Indeed the term phenocopy was introduced to denote heat-shock-induced malformations that were very similar to known genetically determined traits. German (1984) has proposed that heat shock to the mammalian embryo during the critical period of organogenesis can alter the established programme of gene activation and inactivation essential for intrauterine development. At the cellular level, heat shock certainly has effects that would have consequences for normal mammalian development. For example, although heat shock will induce cell cycle progression delay (Dewey & Holden, 1984), it can also enhance the stimulatory effect of certain growth factors on quiescent murine cells (Van Wijk, 1984). Other effects on differentiation have been reported. For instance, heat shock will inhibit the dimethyl sulphoxide-induced differentiation in the murine erythroleukaemia system (Raaphorst *et al.*, 1984) and hormonal responsiveness in *Xenopus* liver cells (Wolffe *et al.*, 1984).

**Heat shock proteins and viral infection**

During the lytic infection of monkey and mouse cells with simian virus 40 or polyoma virus, there is marked increase in synthesis of host proteins of 92 kDa and 72 kDa. These proteins are also heat-inducible in uninfected cells (Khandjian & Turler, 1983). Herpes simplex virus infection of hamster cells also causes the accumulation of a heat shock protein of 57 kDa (La Thangue *et al.*, 1984). Infection of Hela cells with adenovirus increases the expression of hsp genes, particularly an hsp 70 gene (Kao & Nevins, 1983). mRNA for this host gene increases in abundance some 100-fold during a wild type adenovirus infection but does not increase more than 2-fold during an infection with virus that lacks E1A gene function. The E1A gene product normally induces transcription of early adenovirus genes, suggesting that both virus and host genes may be subject to the same regulation. In the case of herpes virus, although at least two 'immediate early' products are responsible for the activation of transcription from viral 'early' promoters, these appear capable of activating host cell promoters as well (Everett, 1985). Later adenovirus experiments using human cells with a high level of constitutive hsp 70 expression, but adenovirus lacking E1A gene function, show expression of 'early' adenovirus genes in absence of E1A gene product. Thus such host cells may have their own 'E1A-like' activity (Imperiale *et al.*, 1984) which could explain the high constitutive expression.

An interesting aspect of the E1A gene product itself is that it has a certain structural similarity to the oncogenes *myc* and *myb* (Ralston & Bishop, 1983). The *myc* gene product has been localized to the nucleus (Abrams *et al.*, 1982) where it is possibly involved in the regulation of gene expression. Certainly the level of expression of certain hsp 70 class genes is elevated in several tumour lines (Imperiale *et al.*, 1984), implying that some cellular function can stimulate hsp 70 gene activity. Thus it may be revelant that Kingston *et al.* (1984) report that the gene product of a rearranged mouse c-*myc* gene is capable of stimulating expression of chimaeric genes containing a *Drosophila* hsp 70 promoter sequence located more than 200 bases upstream from the transcription start.

The increased synthesis of hsps during viral infection could of course be simply part of a non-specific induction of host genes. However, specific host-coded hsps may have a crucial role in viral replication. The extensive homologies apparent between *E. coli*, *Drosophila* and human hsp 70s (Hunt & Morimoto, 1985) certainly suggest important functions. The function of the *E. coli* protein, *dnaK* protein, is known to be related to host DNA synthesis. Possibly more important is the observation that it appears to be essential for the replication of bacteriophage $\lambda$ in *E. coli* (Zylicz & Georgopoulos, 1984). Whether mammalian hsp 70 proteins are required for mammalian virus replication is not yet known; however, brief heat shock of host cells before viral infection inhibits the subsequent replication of herpes and pseudorabies virus, but not RNA viruses such as EMC or influenza (Gharpure, 1965). Possibly hsp induction has to be carefully timed in relation to virus replication.

**Function of heat shock proteins**

From the preceding sections on developmental regulation of hsp gene expression, it might reasonably be anticipated that hsps play an important role during development. Analysis of mutants certainly indicates a role for hsps and cognate hsps in growth control in yeast (Craig & Jacobson, 1985). Also there is the specific requirement of the *dnaK* gene product in $\lambda$ phage chromosome replication to consider.

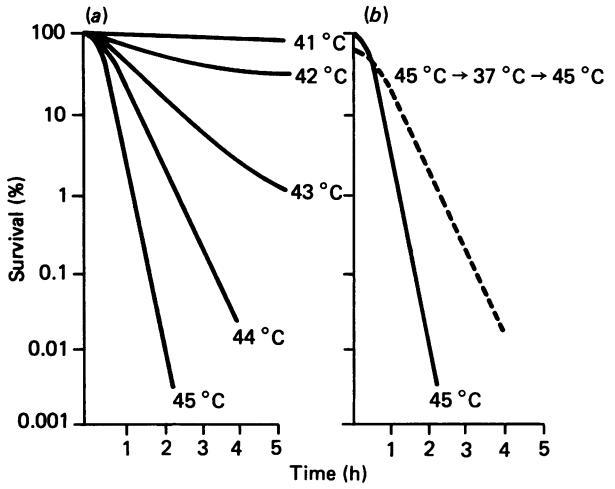


**Fig. 3. Schematic mammalian cell survival curves illustrating the phenomenon of thermotolerance**

(*a*) Illustrates the normal survival curve at various supraoptimal temperatures; (*b*) illustrates the normal survival curve at 45 °C (——) and the survival curve with reduced slope that is obtained if the cells are pretreated by heating at 45 °C for 20 min followed by recovery at 37 °C for 3 h, before return to 45 °C (– – – –).

Other data from a wide variety of organisms nevertheless suggest a role in the development of thermotolerance, or increased resistance to the stress of elevated temperature. Direct evidence comes from mutant analysis in *Dictyostelium*, which indicates the involvement of small hsps in the development of thermotolerance (Loomis & Wheeler, 1982). Mutations in the heat-inducible hsp 70 gene of yeasts were found to confer temperature-sensitive growth in yeast (Craig & Jacobson, 1984).

Work with mammalian cells shows that their tolerance to thermal damage can dramatically increase after prior heat conditioning. It is apparent that the thermal history, the heat fractionation interval and the recovery conditions, all significantly modify the degree of thermotolerance exhibited (Gerwick, 1985). However, there are two main forms of thermotolerance in mammalian cells. During continuous heat treatment, at less than approximately 42.5 °C, heat survival curves show a biphasic response, the cell becoming more heat resistant after a few hours of hyperthermia (see Fig. 3*a*). An alternative form results from an acute heat treatment at temperatures approximately between 43 °C and 45 °C (see Fig. 3*b*). Under these conditions tolerance does not actually develop. Instead it needs time at 37 °C to express itself. In both situations however the thermotolerance is transient, decaying over a period of time depending on a number of factors.

By following the synthesis and degradation of heat shock proteins in mammalian cells during development and decay of thermotolerance, strong circumstantial evidence was obtained (Landry *et al.*, 1982) that hsps are involved in the acquisition, maintenance and decay of thermotolerance. The levels of certain heat shock proteins in murine tumours (Li & Mak, 1985) and Chinese hamster fibroblasts (Li & Werb, 1982), particularly the hsp 70 class, have been reported to correlate with thermotolerance. On the other hand, Omar & Lanks (1984) compared normal and SV40-transformed mouse embryo cells. The transformed cells had higher basal hsp levels and consistently synthesized the major hsps at a higher rate, both at physiological temperature and after exposure to heat shock (43–45 °C). Parallel determination of cell survival showed that the transformed cells were nevertheless more susceptible to killing by hyperthermia than their normal counterparts. Thus it appears that the higher intrinsic resistance of the normal cells to killing by heat is not directly related to basal hsp levels, nor to the degree to which synthesis of these proteins is induced following hyperthermia. Heterogeneity in induced heat resistance and its relation to hsps is also observed in rat tumour cell clones (Tomasovic *et al.*, 1984).

A complication in these studies is that certain cell lines have a higher intrinsic heat resistance than other cell lines. Such resistance is genetically inherited and those cells are referred to as 'heat-resistant' as distinct from the normal 'heat-sensitive' cell lines. Thermotolerance can however be induced in both 'heat-resistant' and 'heat-sensitive' cell lines following hyperthermic treatment. Whilst the thermotolerance induced in the 'heat-sensitive' cell is quantitatively greater than that induced in the 'heat-resistant' cells, the final level of heat resistance attained after the induction process is nevertheless highest in the 'heat-resistant' cell lines. Thus some sort of overlap may exist between mechanisms of thermotolerance induction and genetically inherited resistance. Although heat-sensitive and heat-resistant melanoma cells have equal amount of hsps (Anderson *et al.*, 1984), Chinese hamster cells show elevated levels of 70 kDa hsps associated with both inherited heat resistance and with induced thermotolerance (Laszlo & Li, 1985). Despite these complexities it would certainly be premature to discount a role for hsps in the development of thermotolerance, although an argument against a specific role for heat-induced hsps emerges from work done on rat hepatoma cells incubated in $Ca^{2+}$-free medium (Lamarche *et al.*, 1985). These cells become refractory to the heat induction of hsps but highly resistant to severe hyperthermia. Moreover, these experiments imply that a $Ca^{2+}$-dependent metabolic process is involved in the generation of the signal for heat induction of hsps.

In order to approach the problem of how hsps might function, localization studies have been carried out. Initially these involved autoradiographic and cell fractionation procedures (Velazquez *et al.*, 1980; Arrigo *et al.*, 1980; Burdon *et al.*, 1984). More recent studies have also employed immunocytochemical techniques. In mammalian cells, although low levels of hsp 72 can be detected in the cytoplasm at normal growth temperature, large amounts are found in the nucleus and nucleolus after heat shock (Welch & Feramisco, 1984). This appears general for the 'hsp 70 class' (La Thangue, 1984; Ungewickell, 1985; Subjeck *et al.*, 1983) but the hsp 84 remains cytoplasmic even after heat shock. Although hsp 95 is basically concentrated in the Golgi apparatus, some is found in the nucleus after heat shock (Welch *et al.*, 1982).

The small hsps (15–30 kDa, Table 1) show the general property of reversible binding to nucleoskeleton during heat shock (Arrigo & Ahmad-Zadeh, 1981; Loomis & Wheeler, 1982; Key *et al.*, 1982). They also tend to aggregate (Arrigo *et al.*, 1985) and such aggregates are readily visible in plant cells as cytoplasmic 'heat shock

granules' (Neumann *et al.*, 1984). In plants, hsp 21 and hsp 27 are also transported into chloroplasts (Vierling *et al.*, 1986; Kloppstech *et al.*, 1985). Localization studies can only suggest and possibly exclude certain types of intracellular functions. More detailed studies indicate particularly intimate associations of hsp 70 with plasma membrane glycoproteins (Hughes & August, 1982), with microtubules (Lim *et al.*, 1984), with intermediary filaments (Wang *et al.*, 1980) and with microfilaments (La Thangue, 1984) as well as with the nuclear matrix (Pouchelet *et al.*, 1983), nuclear heterogeneous ribonucleoprotein particles (Kloetzel & Bautz, 1983) and nucleoli (Pelham, 1984). Such associations may be critical in recovery from heat shock. For example, Pelham (1984) has produced evidence that hsp 70 aids in the repair of heat damaged nucleoli, and suggests that this is achieved by binding to exposed hydrophobic surfaces. A model has been proposed in which repeated binding and ATP-driven release of hsp 70 help to solubilize hydrophobic precipitates of abnormal or denatured proteins (Lewis & Pelham, 1985).

Hsp 84, which remains cytoplasmic, may have quite a different, and possibly regulatory, role. For example, it is to be found in association with several steroid receptors (Catelli *et al.*, 1985; Schuh *et al.*, 1985; Sanchez *et al.*, 1985) as well as with the Rous sarcoma virus transforming protein, $pp60^{v\text{-}src}$ (Schuh *et al.*, 1985).

Other properties of hsps have nevertheless been described which make general conclusions difficult with regard to cellular functions. Hsp 73 shows specific affinity for poly(A) (Schonfielder *et al.*, 1985). Hsp 69 interacts with the cellular tumour antigen p53, which is known to complex with the large-T antigen of simian virus 40 (Pinhasi-Kimhi *et al.*, 1986), and proteins of the hsp 70 group are related to proteins involved in the dissociation of clathrin triskelia from coated vesicles (Ungewickell, 1985). More specifically, an hsp 48 of yeast turns out to be an isoprotein of enolase (Ida & Yahara, 1985) and as already mentioned a very small hsp of 8 kDa has been identified as ubiquitin in avian cells (Bond & Schlesinger, 1985).

## Applications of heat shock studies

**Biotechnical applications.** An understanding at the molecular level of the results of thermal stress and of thermotolerance may be of considerable biotechnological importance. Although heat shock will induce tolerance to further heat stress it also leads to increased tolerance to other stresses. For example, the application of heat stress to yeast greatly increases their subsequent tolerance of ethanol (Watson & Cavicchioli, 1983). Heat shock of hamster cells also increases their resistance to ethanol (Li *et al.*, 1980) but conversely exposure to ethanol, an hsp inducer (Table 2), increases the resistance of hamster cells both to heat shock and to drugs such as adriamycin (Li & Hahn, 1978). Clearly the range of stress protection possible remains to be fully explored, but if plants could be genetically engineered to withstand heat as well as other stresses, possibly through increased expression of hsps, this would be of considerable economic value. Animal cells in culture also have a key role to play in biotechnological development. In bulk culture animal cells capable of withstanding considerable 'stress' will be required if high yields are to be achieved. High yields may in turn lead to higher temperatures in culture vessels. However if expression vectors, incorporating heat shock promoter elements, were transfected into such cells, then the excess heat might be utilized to induce the transcription of specific genetic elements at the end of the growth period.

A potential difficulty concerns the expression of foreign gene constructions in eukaryotic, or prokaryotic, cells. To these cells the products of such genes might appear 'aberrant' or 'abnormal'. For this reason such products might then elicit the activation of hsp genes, and amongst the hsps are known elements of protein degradative systems (e.g. the ubiquitin proteolytic system in eukaryotes, and the Lon system in *E. coli*). Of course, by judicious use of promoters and other genetic regulatory sequences in expression vectors, there may be such large amounts of foreign gene product synthesized that the effects of proteolysis are minimal by comparison. Nevertheless, steps to eliminate hsp induction may be necessary to ensure maximal expression of foreign genes.

**Clinical applications.** Heat treatment is being developed as an anticancer modality (Hahn, 1982). Human and other mammalian tumour cells will die after hyperthermia corresponding to only a modest increase of 4–8 °C above normal. The efficiency of the process depends amongst other things on the length of the treatment as well as the temperature experienced (Fig. 3*a*). On the other hand the design of clinically useful anticancer treatment procedures will almost certainly depend on fractionated regimes. To be successful this will require a basic understanding of the biology of fractionated heating procedures as well as the development of the thermotolerance that will ensue. This will provide clear answers with regard to optimal recovery times and suitable intervals between heat treatments. Unfortunately the development of thermotolerance (Fig. 3*b*) is a major impediment to the effective application of clinical hyperthermia (Gerwick, 1985). Chemical agents related to benzaldehyde are now being developed for clinical use in order to increase the cell killing powers of hyperthermia and to eliminate the development of thermotolerance (R. H. Burdon, unpublished work).

As a final comment one cannot help wondering whether the genes and the proteins involved in the complexities of the heat shock response may be important in some, as yet unidentified, animal disease states, and that medically significant mutations that affect heat shock loci exist. On the other hand, it is quite possible that this system of proteins is simply so important that variation is incompatible with life.

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